Investigating local properties of living cells with the atomic force microscope

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Introduction

The atomic force microscope (AFM) provides a novel tool for investigating the local properties of biological surfaces, and manipulating living cells (for a review see Henderson, 1994). It has recently become clear that when imaging living cells with the AFM the local visco-elastic properties contribute significantly to the image contrast (Henderson et al., 1992; Chang et al., 1993; Fritz et al., 1994; Schoenenberger and Hoh, 1994; Putman et al., 1994). This has allowed intracellular structures to be visualized 'through' the relatively soft plasma membrane of cells. Further, the cells appear to survive the repeated interaction with the AFM tip such that dynamic processes such as the movement of actin stress fibers or vesicle trafficking (Henderson et al., 1992; Fritz et al., 1994; Schoenenberger and Hoh, 1994) can be visualized. At present the visualization of intracellular structures is thought to result from the deformation of the plasma membrane induced by the tip. However, it has also been suggested that the tip actually penetrates the membrane during imaging. That the tip under some conditions can rupture the membrane is clear, although the exact conditions for such rupture have not been determined. We have begun an effort to exploit the sensitivity of the AFM to visco-elastic properties with goal of producing quantitative maps of the micromechanical properties of living cells with subcellular resolution. Here we review some recent results AFM studies of Madine Darby Canine Kidney (MDCK) cells, and also present data showing the mechanical perturbation of the monolayer and subsequent cellular repair.

Results and Discussion

MDCK cells are an excellent system for atomic force microscopy (AFM). These cells form homogenous monolayers of polarized cells that are well attached to the underlying substrate and height variations along the apical surface are typically 2-3 μm (Fig. 1). Topographs of the apical cell surface reveal two types of surface protrusions, smooth bulges and spikes, in addition to cell boundaries and nuclei (Hoh and Schoenenberger, 1994). Both spikes and bulges exhibit dynamic behavior over the time scale of minutes to tens of minutes (Schoenenberger and Hoh, 1994). We have previously suggested that the spikes result from a filamentous structure abutting the apical membrane, such that the membrane 'tents' itself over the filament and is swept in the scan direction (Hoh and Schoenenberger, 1994). Comparison of images recorded at different forces shows that forces above 2 nN are required to achieve good contrast. This is consistent with the view that most of the detailed morphology seen in these images results from the apical membrane deforming around intracellular structures.

Evidence of cell viability during and after imaging is provided by the dynamic behavior of the cells over time (Schoenenberger and Hoh, 1994). Cellular extensions move over the time course of hours and the repair of damage to the plasma membrane inflicted by the tip still occurs after imaging cells for several hours. The viability of cells was also confirmed by returning individual cover slips after imaging to culture conditions for another 12-24 hours, and staining with trypan blue. Only scattered dead cells could be seen in the monolayer.

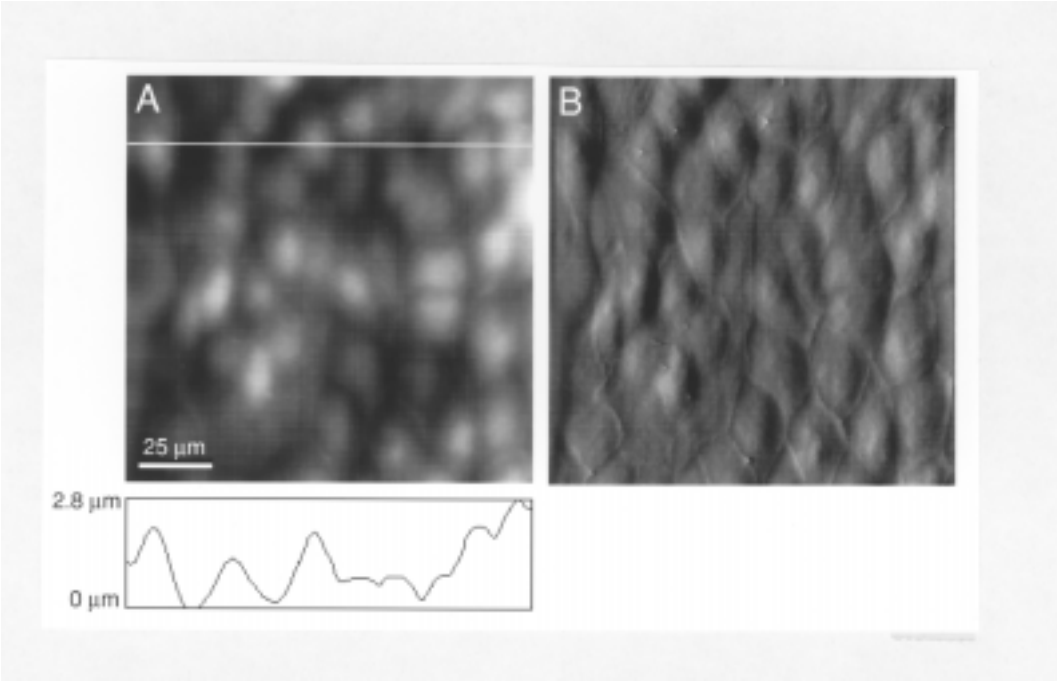

Figure 1. AFM images of a living MDCK monolayer. Cells were cultured and imaged as previously described (Hoh and Schoenenberger, 1994). A. Height image with cross section. B. Error signal image.

The mechanical properties of the apical membrane can be probed by pushing the AFM tip against the membrane and collecting a force curve. These curves show that the membrane deforms approximately 500 nm per nanoNewton of applied force. When converted into a stiffness curve, shown in Fig. 2, it is clear that the membrane has an effective spring constant that is at least two orders of magnitude smaller than the cantilever. The stiffness of the membrane at ~300 nm indentation is less than the noise level of these measurements (~0.001 N/m). These values are very close to those reported by Weisenhorn et al. (1993) who estimated a Young's modulus of 0.013-0.15 MPa for lung carcinoma cell line. We have also examined the effect of scan rate on the shape of the approaching force curve and found that there is no significant effect of increasing the rate several fold over 4 µm/s, the acquisition rate for the curve shown in Fig. 1. This suggests that the response in this curve is primarily elastic, although an active contribution by the cell can not be excluded. These measurements are also consistent with the observation of Putman et al. (1994) who find that relaxation time for the indentation of an AFM tip on a monkey kidney cell is 200-300 ms. It is important to note that the measurement described here were made with a standard pyramidal AFM tip and that the geometry of the tip will significantly influence the stiffness measurements.

The stiffness of the cells in the monolayer can be manipulated by fixation with glutaraldehyde. This change in stiffness can be monitored in real time with a temporal resolution of <10 seconds (the time between force curves). Fig.3 shows a series of force curves collected on an MDCK monolayer during fixation. It shows that the fixation appears to be complete in <80 seconds, as judged from the mechanical response. There is also a significant roughing of the surface during the fixation (not shown).

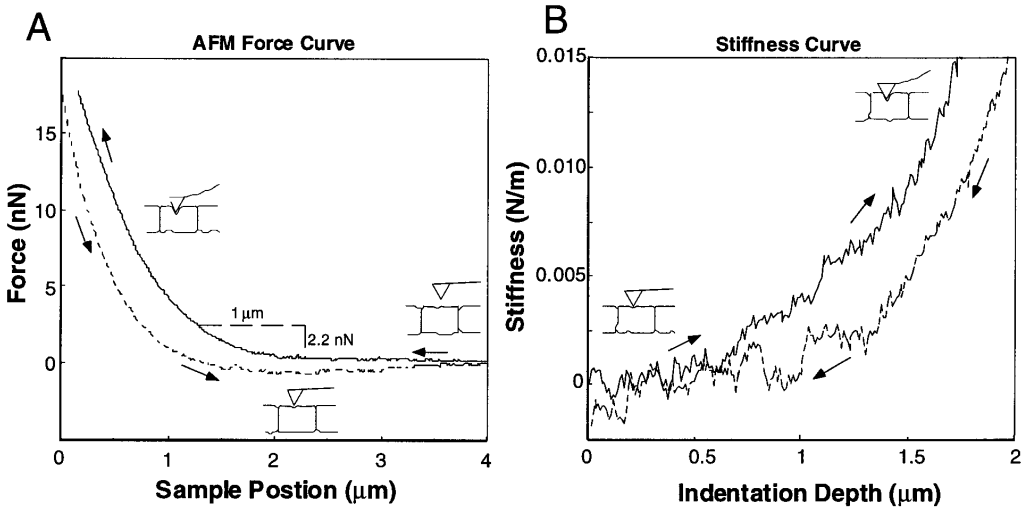

Figure 2. Stiffness of an MDCK monolayer. A. A conventional force curve was acquired using in pyramidal silicon nitride tip on a cantilever with a calibrated force constant of 0.06 N/m. B. Stiffness curve, in which the stiffness S is defined as S=dF/dD, where F is force and D is indentation depth. The force curve was converted to a stiffness curve by first making an indentation curve as described by Weisenhorn et al. (1993), in which the deflection of the cantilever is plotted versus the indentation depth into the cell. The first derivative of this indentation curve, which shows the slope of the indentation curve at each point, was then calculated to produce the stiffness curve.

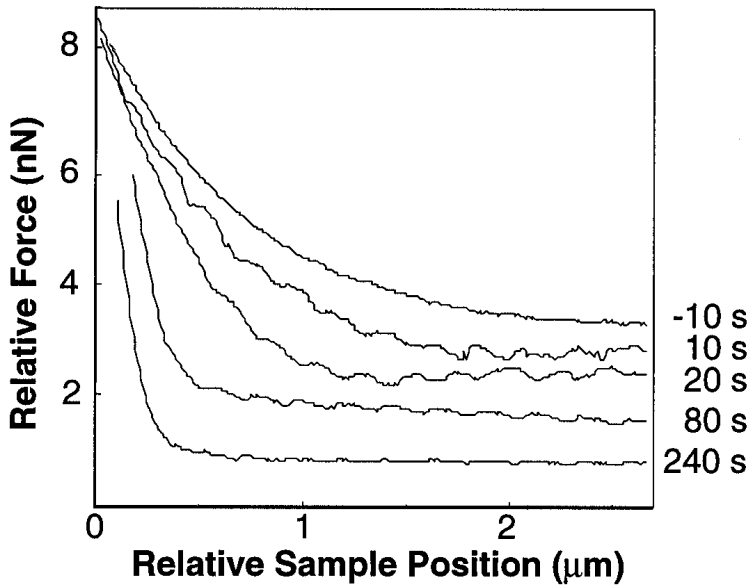

Figure 3. Mechanical response of an MDCK monolayer to glutaraldehyde fixation. Sequential force curves from the monolayer beginning before the addition of glutaraldehyde (-10 sec), continuing over a time period of ~4 minutes. The soft response seen at -10 seconds gradually becomes harder as the cells are fixed. Note that the offsets between the curves are for display purposes only. Therefore all forces and sample positions are relative. Specifically the appearance that the fixation results in a 1-2 μm thinning of the monolayer is not correct. The actual thinning is ~100 nm.

In addition to probing properties of cells, the AFM tip can be used to manipulate the cell surface (Parpura et al., 1993). We have used the AFM tip to inflict the damage to a specific location on a single cell with in a monolayer of MDCK cells and directly monitor the repair (Fig. 4). To achieve this a 5x5 μm area within the boundary of a single cell was selected. The imaging force over this area was increased to the maximal available, approximately 10 nN, and the scan frequency was increased to 10-20 Hz. After several frames the surface became very flat and, as indicated by force curves, very hard, suggesting that the glass surface had been exposed. The microscope was then returned to the parameters used for imaging and a larger area, including the damaged area, was imaged. This process resulted in a hole in the cell that apparently extended through the entire monolayer. After withdrawing the tip the hole closed completely in ~40 minutes, suggesting that the cell had repaired the damaged membrane. However, repair was slowed or even prevented if imaging proceeded immediately after damaging the cell and the tip had to be withdrawn for extended periods in order for the hole to close.

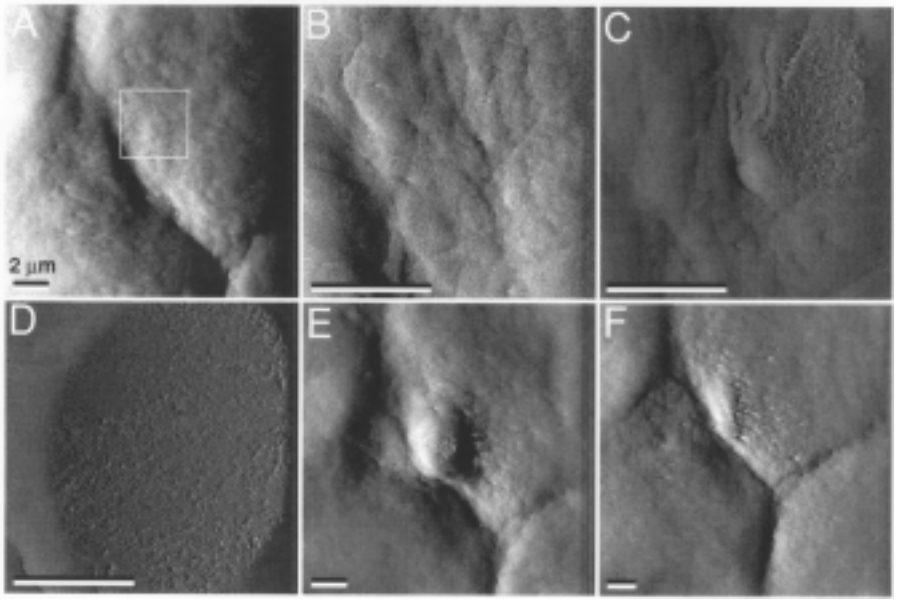

Figure 4. Local mechanical damage to the monolayer inflicted by the AFM tip, and imaging of the repair. A. Monolayer of MDCK cells. The ridge that runs diagonally across the image is the boundary between two cells. The box indicates the area shown in B-D. B. Cell surface before tip inflicted damage. C. The imaging force and scan rates are increased a rupture occurs in the cell surface exposing a small hole. D. As the hole in the cell opens up the flat glass surface becomes clearly visible. E. A lower magnification image shows the hole within a cell. F. 40 minutes later the hole has been repaired. Note the large number of spikes in the surface at the site the damage.

The micromechanical properties of cells have been studied in a number of systems (for reviews see McNeil, 1993; Tran-Son-Tray, 1993). However, the approaches used so far do not have lateral resolution that allows subcellular features to be examined. The results presented here demonstrate that the AFM will allow measurements previously made at the level of tissues and whole cells to be extended to micromechanical maps of individual cells. Further, the tip of the AFM can be used to mechanically manipulate living material and under controlled conditions produce ruptures in the plasma membrane and allow the study of the process by which cells repair such damage.

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